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MANGROVE ECOSYSTEMS

A MANUAL FOR THE ASSESSMENT OF BIODIVERSITY

A follow up of the
National Agricultural Technology Project
(NATP.), ICAR.

Mangrove Ecosystem Biodiversity :
Its Influence on the Natural Recruitment of
Selected Commercially Important Finfish and Shellfish
Species in Fisheries

Edited by :
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Sediment Analysis

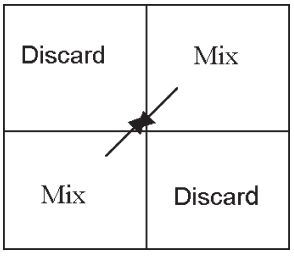
D. Prema, K. S. Leela Bhai and P. K. Jayasurya

1. SEDIMENT SAMPLING AND PROCESSING

Sediment sampling:

Sediment sample is collected from the stations (seaside, river mouth and within the Mangroves, depending on the topography and area and upper reaches of river) using a grab.

Sample should be representative of the area sampled. For this, collect samples from at least four sites in a station. Pool these samples in a wide basin. The pooled sediment is mixed thoroughly. Then quartering is done, removing the opposite quarters as shown below.



Quartering of sediment sample

Quartering is done till a sizeable quantity i.e., around 500 g sediment is obtained. Collect in heavy-duty plastic bags.

Sediment processing:

Do not store the sediment wet for more than one day. Wet samples should be kept under refrigeration if storage is needed in the wet stage for more than one day. Then air-dry the sediment in shade in well-ventilated places. To reduce drying time, oven drying can be done at 50-60°C. (Temperature should not be increased above 60°C since it will cause loss of nutrients). Then pulverize the sediment gently by breaking clods using a pestle and mortar. (Do not over grind the sediment so as to break the sand particles, which will cause errors in textural analysis). Then sediment samples can be stored in well-labeled, capped plastic bottles or heavy-duty plastic bags. At this stage, it is ready for analysis. While storing, store in a dry place.

2. HYDROGEN ION CONCENTRATION (pH)

Principle:

The degree of acidity or alkalinity in soils also known as soil reaction is determined by hydrogen ion (H^+) concentration in soil solution. An acid soil has more H^+ than OH^- ions, whereas basic or alkaline solution contains more OH^- ions than H^+ ions. To categorize these conditions, the term soil pH is used.

Determination of dry pH of sediment

Procedure:

Calibrate the pH meter with buffer solution according to the maker's instructions and wash the electrode well.

To a 10 g air dried sediment sample add 25 ml water. The suspension is stirred at regular intervals for 20-30 min. Then pH is measured with glass electrode. The suspension is stirred just before electrode is immersed.

Determination of wet pH of sediment

Procedure:

Wet pH is to be determined before air-drying the sediment. After calibrating the pH meter, insert the electrode of the pH meter directly in to a wet lot of sediment sample and the pH recorded is the wet pH of the sediment.

3.Oxidation Reduction Potential (Eh) (Redox ptential)

Oxidation Reduction Potential (Eh) is a quantitative measure of the tendency of a system to oxidize or reduce susceptible points. When Eh is positive and high it is an oxidizing system. When Eh is negative and low it is a reducing system.

Oxidation Reduction Potential of a fresh sediment can be determined using an ORP meter or using a pH meter with a compatible ORP electrode. After calibrating the ORP meter or the pH meter with the ORP electrode, using the suitable ORP buffer, wash the electrode well. Then insert the ORP electrode into the fresh sediment and note the reading of Eh.

4. SALINITY

Principle:

When standard silver nitrate solution is added to the soil extract, the chlorides will be precipitated as silver chloride. When all the chlorides being precipitated, next drop of silver nitrate will react with potassium chromate giving red colour of silver chromate.

Reagents:

(1) Silver nitrate (0.144 *N*): Dissolve 24.5 g of silver nitrate in 1 litre water.

(2) Indicator: 10 % potassium chromate.

Procedure:

Weigh 5 g of sediment and transfer it in to a conical flask. Add 25 ml distilled water and shake it for 30 minutes. The sample is then filtered through No.42 filter paper. To 10 ml of the filtrate is added 4 drops of 10 % potassium chromate indicator. This is then titrated against silver nitrate. The end point is the colour change from pale yellow to pale pinkish red. The salinity of the filtrate can alternatively be determined using appropriate probe of a salinometer or multiparameter instrument after calibration.

Calculations:

1 ml of 1*N* Ag NO_3 = 35.46/ 1000 g of Cl

$$\text{Salinity} = \frac{X \times 0.144 \times 35.46 \times 25 \times 1000}{10 \times 5 \times 1000} \text{ g/kg}$$

$$= \frac{X \times 0.144 \times 35.46 \times 25}{10 \times 5} \text{ mg/g or ppt}$$

where

X = volume of $AgN0_3$ used
0.144 = normality of $AgN0_3$
35.46 = eq.wt of Cl
25 = distilled water used for extraction
10 = volume of extractant used for titration
5 = quantity of sediment taken.
1000 = for converting 35.46mg to g
1000 = for converting g to kg

Interstitial salinity : Interstitial salinity can be determined by inserting the salinity probe of a salinometer or multiparameter instrument (after calibration) into the fresh sediment.

5. SOIL TEXTURE

(Mechanical Analysis by International Pipette Method)

Principle:

Mechanical analysis is defined as the analytical procedure by which individual particles are separated to determine the size distribution of the soil. According to the relative proportion of various sizes of the individual particle, they are separated in to various groups and is known as textural classification of soil. According to International society of soil science system, the mechanical separates of soil are classified as follows.

Soil separate	Diameter limit (mm)
Coarse sand	2 to 0.2
Fine sand	0.2 to 0.02
Silt	0.02 to 0.002
Clay	< 0.002

Mechanical analysis consists essentially of two distinct operations:

1) Dispersion: The most important cementing agents are organic matter, colloidal clay and dehydrated colloidal oxides of Fe, Al.

   The important physical techniques that have been used to effect the mechanical disruption of aggregates in to completely dispersed particles are shaking, stirring and boiling etc.

The chemical aid in soil dispersion are based on

(a) Oxidation of organic matter by H_2O_2; (b) Removal of flocculating ions by introducing single cations; (c) Peptization of colloidal particles through the introduction of ions that increase negative potential.

2) Fractionation of the sample in to various separates: The relation between the particle size and its rate of fall through a fluid is expressed by Stokes law as follows:

$$V = 2gr^2(dp-dw)/9\eta$$

Where, V = velocity of fall of particles, r = radius of particles, dp = density of particles, dw = density of medium, η = coefficient of viscosity of liquid and g = acceleration due to gravity.

Pipette method:

According to this method, instead of completely separating the fractions of the mechanical compositions, suspension samples are taken from the different depths subsequent to shaking, after the lapse of certain predetermined period of settling which are dependent on the dimensions of the mechanical elements and on the temperature of water.

Procedure:

To the wet sediment add 10 ml of 1*N* HCl and 10 ml of 6% H_2O_2. Stir it till the effervescence stops. Add 100 ml of distilled water and allow settling. The supernatant liquid is decanted after one day. The process is continued for several times till the supernatant liquid is chloride free. The sediment is then air dried, pulverized and sieved through a 2mm mesh sieve to obtain the size fraction of sand and finer particles. Weigh 20 g of the sieved sediment and it is wet sieved into 500 ml sedimentation cylinder using a 230 mesh sieve. Sediment retained in the sieve is dried and weighed to obtain the sand fraction. Add 10 ml of 1*N* NaOH to the cylinder and make up to 500 ml. The cylinder is shaken for 10 minutes. Draw 10 ml of suspension using a pipette at a depth of 20 cm after 4 minutes into a weighed petridish. Dry it at 105º C to constant weight. Difference in weight will give the quantity of clay + silt in 10 ml suspension. The whole suspension is again shaken and kept undisturbed. After 6 hrs. and 15 minutes, draw 10 ml suspension in a similar manner as described earlier in a weighed petridish and then dry at 105ºC until constant weight is attained.

Calculation:

(a) % of sand = Wt. of sand x 100 / 20

(b) Silt + clay fraction:

Total volume of suspension = 500 ml

Volume of suspension taken = 10 ml

% silt + clay = (Wt of silt + clay) x 500 x 100 /10 x20

(c) Clay Fraction:

% Clay = Wt. of clay x 500 x100/10 x 20

(d) % silt = (% silt +clay) - % clay

6. ORGANIC CARBON

(Walkley and Black's titration method)

Principle:

The soil is digested with potassium dichromate solution and sulphuric acid making use of the heat of dilution of sulphuric acid. The excess of potassium dichromate not reduced by the organic matter of the soil, is determined by titration with standard ferrous sulphate or ferrous ammonium sulphate solution.

Reagents:

1) Potassium dichromate 1*N*: Dissolve 49 g of potassium dichromate in water and make it to 1 litre.

2) Sulphuric acid + silver sulphate: Dissolve 25 g of silver sulphate in 1 litre of conc. sulphuric acid.

3) Phosphoric acid (85%)

4) Diphenylamine indicator solution: Dissolve 0.5 g of diphenylamine in a mixture of 100 ml sulphuric acid and 20 ml water and store in a colored bottle.

5) Ferrous ammonium sulphate (*N*/2): Dissolve 196 g of A.R grade $FeSO_4 (NH_4)_2SO_4. 6H_2O$ in water. Add 20 ml sulphuric acid and dilute to 1 litre.

Procedure:

Take 0.5 – 2 g of soil (0.5 mm sieved) in 500 ml conical flask. Add 10ml of 1*N* potassium dichromate and 20 ml conc. H_2SO_4. Shake well for a minute or two and allow it to stand for about 30 minutes. Add 200 ml water, 10 ml phosphoric acid and 1 ml diphenylamine indicator solution. A deep violet color will appear. Titrate with *N*/2 ferrous ammonium sulphate solution, till the violet color changes to blue and finally to green. In the same way carry out a blank determination also and calculate the results as follows:-

Calculation:

Weight of soil taken = W g

Volume of 0.5 *N* ferrous ammonium sulphate required for reducing 10 ml $K_2Cr_2O_7$ solution (Blank Reading) = X ml

Volume of 0.5 *N* ferrous ammonium sulphate required for reducing the excess of dichromate (experimental reading) = Y ml

Difference = (X–Y) ml

1 ml of 1*N* $K_2Cr_2O_7$ = 0.003 g carbon

$$\% \text{ of carbon in soil} = \frac{(X-Y) \times N \times 0.003 \times 100}{W}$$

Where N = normality of ferrous ammonium sulphate

7. Extraction of ammonia, nitrate and nitrite in sediments

The ammmoniacal nitrogen, nitrite nitrogen and nitrate nitrogen can be together extracted with one reagent ie, 2*M* KCl solution.

Reagent:

2 *M* Potassium chloride solution:

Dissolve 150 g of KCl in 1000ml of distilled water

Procedure :

Shake 15g fresh sediment in 150ml of 2 *M* KCl for 1 hour and allow to settle. Filter the supernatant through Whatman no.1 filter paper. The filtrate can be used for determination of ammoniacal nitrogen, nitrite nitrogen and nitrate nitrogen.

7a. Determination of NH_3^-N

(Phenol hypochlorite method)

Principle:

In this method phenol and hypochlorite react in an alkaline solution to form phenyl quinone-monoimine, which in turn, react with ammonia to form indophenol. Indophenol gives the solution a blue colour, the intensity of which is propotional to the concentration of ammonia present. Sodium nitroprusside is added to intensify the blue colour. Both ammonia and ammonium are measured because in a strong alkaline solution, all the ammonium is converted to ammonia. This procedure gives an estimate of total ammonia nitrogen.

Reagents :

1. Phenol-alcohol solution: Dissolve 10g of reagent grade phenol in 100ml of 95% v/v ethyl alcohol, U.S.P or methyl alcohol.
2. Sodium nitroprusside 0.5%: Dissolve 1g of sodium nitroprusside in 200ml of water.
3. Alkaline solution: Dissolve 100g of trisodium citrate and 5 g of sodium hydroxide in 500ml of water.
4. Sodium hypochlorite solution : Use a solution of commercial hypochlorite, which should be atleast 1.5*N*.
5. Oxidising solution: Mix 100ml of sodium citrate solution (alkaline solution) and 25ml of hypochlorite solution and use the same day (1:4 ratio – sodium hypochlorite : alkaline solution)
6. Stock standard solution: 3.147g ammonium chloride dissolved and made up to 1000ml. This gives a 1000 ppm solution of NH_3 N.

Procedure:

The procedure consists of the successive addition of 2ml of phenol solution, 2ml of nitroprusside solution and 5ml of oxidizing solution to 50ml of sample mixing thoroughly after each addition . The colour is allowed to develop at room temperature (22 -27ºC) for 1hr and the absorbance is recorded at 640 nm in a spectrophotometer. Correct the absorbance with that of the reagent blank.

Preparation of Calibration graph:

Dilute the standard stock solution to get working standards of 0.1, 0.2, 0.3, 0.4, 0.5 ppm of $NH_3 – N$ concentrations. Follow the above procedure and measure the absorbance at a wavelength at 640 nm in a spectrophotometer and draw a calibration graph.

Calculation:

Obtain the concentration (ppm) of $NH_3 – N$ in the sample from the calibration graph.

NH_3^-N = ppm x150/15

Note:

1. All the reagents are to be prepared using ammonia free distilled water.

   All the glasswares used must be cleaned by washing initially with warm dilute hydro-chloric acid and rinsing thoroughly with distilled water.
2. If the strength of hypochlorite is not satisfactory, a fresh reagent should be used for analysis.

7b. Determination of NO_3 and NO_2 :

Nitrate

Reagents:

1. Phenol solution:

   Dissolve 46g of dry A.R. Quality phenol in 1000ml of distilled water. It is stored in a glass bottle tightly stoppered.
2. Sodium Hydroxide:

   Dissolve 29 ± 0.5 gms of A.R. quality Sodium Hydroxide in distilled water. Cool and dilute to 2000 ml.
3. Buffer Reagent:

   Pipette out 25ml of Phenol solution into a dry beaker and add 25ml of sodium hydroxide solution. The solution is stable for one hour.

4. Copper Sulphate Solution:

   Dissolve 0.1 g of A.R. copper sulphate in 1000ml of distilled water.

5. Hydrazine Sulphate Solution:

   Dissolve 14.5 g of A.R. quality hydrazine sulphate in 2000ml of distilled water. Store in a dark glass bottle. The solution is stable for one month.

6. Reducing Agent:

   Mix 25 ml of copper sulphate solution and 25ml of Hydrazine sulphate solution in 50ml measuring cylinder. The solution is stable for one hour.

7. Acetone:

8. Sulphanilamide Solution:

   Dissolve 5 g of Sulphanilamide in a mixture of 50 ml concentrated Hydrochloric acid and about 300ml distilled water. Dilute to 500 ml with water. It is stable for a few months.

9. N1-Naphtyl Ethylene Diamine Di-Hydrochloride Solution (N.N.E.D):

   Dissolve 0.5 g of N.N.E.D. in 500ml distilled water. Store the solution in a dark bottle.

10. Standard Nitrate solution:

    Dissolve 1.629g of analytical reagent quality potassium nitrate in 1000 ml. This gives 1000 ppm of nitrate nitrogen. Prepare working standards of 0.02, 0.03, 0.04, 0.05, 0.06 ppm and prepare a calibration graph. From the calibration graph find out the ppm of nitrate nitrogen in the sample.

Procedure:

Measure out 50 ml of the filtrate with a 50 ml measuring cylinder into a 250 ml conical flask. Add 2 ml of buffer reagent and mix. After the buffer has been added to all the samples, add with rapid mixing 1.0 ml of reducing agent and keep the flasks away from sunlight in a dark place for about 20hrs. Add 2ml of acetone, and after 2 min. add 1 ml of sulphanilamide solution. After 2 minutes, but not later than 8 minutes add 1 ml of N.N.E.D solution and mix. Take reading at 545 nm . The calculated ppm is $(NO_3 + NO_2)$. Deduct the NO_2 (ppm) from this to get NO_3 alone.

Calculation

NO_3 alone = $[(NO_3 + NO_2)$ ppm x150/15$] - NO_2$ ppm. x 150/15.

7c. Nitrite

Reagents:

1. Sulphanilamide solution (as in Nitrate method)
2. N1 Napthyl Ethylene Diamine Dihydrochloride (N.N.E.D.) (As in Nitrate method)
3. Standard Nitrite Solution:

Dissolve 1.5 g of A.R. Sodium Nitrite in 1000 ml of distilled water. Store in a dark bottle with 1 ml of Chloroform. This gives 1000 ppm solution of nitrite nitrogen. Prepare working standards of 0.02, 0.04, 0.06,0.08, 0.1ppm and prepare a calibration graph. From the calibration graph find out the ppm of nitrite.

Procedure:

Measure out 50 ml of filtrate in the conical flask. Add 1 ml of sulphanilamide solution to each sample. After 2 minutes, but not later than, 8 minutes add 1 ml of N.N.E.D. solution to each and mix immediately. Measure the absorbance at 545 nm. Carry out the procedure with standard nitrite solution also.

Calculation:

NO_2 = ppm x 150 / 15

8. AVAILABLE PHOSPHORUS

(Olsen's method)

***(0.5 M sodium bicarbonate extraction*)**

Principle:

All soils contain insoluble phosphates mainly di- and tri-calcium phosphates in neutral and alkaline soils and aluminium and ferric phosphates in acid soils. Phosphate ions are present in small concentration in soil solution according to relative amounts of calcium, aluminium and ferric ions. If the concentrations of metallic ions are reduced, concentration of phosphate ions increases in order to maintain various soluble products at their constant values.

An alkaline (pH 8.5) bicarbonate solution can repress the concentration of Ca ions by precipitation as $CaCO_3$ and Al and ferric ions by precipitation as hydroxides. Thus phosphate ion concentrations are increased and available phosphate can be extracted from soil by shaking with alkaline sodium carbonate and filtering. Activated carbon(phosphate free) must be used with most soils to absorb soluble organic matter and it is necessary to allow time for CO_2 bubbles to escape.

Reagents:

1. Extracting solution: 0.5 *M* sodium bicarbonate (pH 8.5).

   Dissolve 420 g sodium bicarbonate to 10 litres, incorporating about 45 ml 5*N* sodium hydroxide to adjust the pH to 8.5 + or – 0.1.

2. Activated carbon, purified:

   Test the carbon for phosphorus by shaking with extractant, filtering and developing molybdenum blue. If measurable amount of phosphorus is obtained, shake the main stock of carbon with extracting solution, filter and wash the carbon well with water, dry in an oven and pulverize to powder. Retest to establish the absence of phosphorus.

3. Stannous chloride (approx 0.5 *M*) (Stock solution):

   (a) Dissolve 10 g stannous chloride in 25 ml Conc. HCl.

   (b) Diluted Solution – Add 1 ml conc. stannous chloride to 66 ml water.

4. Ammonium molybdate – HCl solution: Dissolve 15 g of ammonim molybdate in 400 ml warm water and filter , add 400 ml of 10 *N* HCl slowly with mixing and make it to 1 litre.

Procedure:

Air dry soil should be ground to pass through a 0.5 mm sieve. A 2.5 g soil sample is suspended in 50 ml of $NaHCO_3$ solution of pH 8.5 along with 1 teaspoon of carbon black. Fine suspension is shaken for a period of 30 minutes. The solution is filtered through a Whatman No.40 or other suitable filter paper. A 5 ml aliquot of clear filtrate is pipetted in to 25 ml vol. flask. A volume of 5ml acid molybdate is added and the flask is allowed to stand for the generation of CO_2. After that add 10 ml distilled water, then 1 ml stannous chloride (working solution), by immediate shaking and make up the volume to 25 ml and mix thoroughly. Prepare a blank as above and read the intensity of colour developed, at 660 nm, after 10 minutes and within 20 minutes.

Preparation of standard curve:

Dissolve 0.2195 g KH_2PO_4 in 1 litre $NaHCO_3$ solution. This stock solution contains 50 µg P/ml. Pipette out different quantities of solution from the standard in 25 ml vol. flask, add 5 ml molybdate reagent, add 1 ml diluted stannous chloride. Read the intensity of colour developed after 10 min (660 nm), within 20 minutes.

Calculation:

Plot the absorbance values obtained with standard phosphorus solutions against the amount of phosphorus present. From the graph record the number of micrograms of phosphorus corresponding to the absorbance values given by test solution.

ppm P in soil = ppm P in solution $\times$ 25/5 $\times$ 50/2.5 .

9. AVAILABLE POTASSIUM

Principle:

For most soils the potassium removed is largely that associated with the clay and humus complex as exchangeable ions but in some saline extracts there may be a fair amount of water-soluble potassium. In the assessment of availability, the exchangeable and water-soluble potassium ions are not differentiated, the sum of the two being measured in the soil extract, usually by flame photometry.

It is accurate enough to shake soil with ammonium acetate solution (1:20 ratio) a procedure that removes 90-95% exchangeable potassium and all water-soluble potassium.

Reagents:

1. Extracting solution- ammonium acetate, 1*N* (pH 7.0): Dissolve 77 g of ammonium acetate in 1 litre of distilled water.

2. Standard Potassium-1000 ppm: Dissolve 1.907 g dry potassium chloride in 1 litre distilled water.

Procedure:

Transfer a weight of air-dried soil containing 5 g of oven dried soil to 250 ml flask and add 100 ml ammonium acetate. Shake for 30 min. and filter. Measure the concentration of extracts by flame photometry, calibrating photometer with standards containing 0, 5, 10, 15 and 20 ppm K in 1*N* ammonium acetate.

Calculation:

From graph, let the concentration of soil extract be 'A' ppm K.

Then concentration of potassium in oven dry soil = A x 100/5 ppm